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(54) **Enzyme modified protein and a process for its preparation.**

(57) A process for the preparation of isolated vegetable protein materials includes extracting vegetable protein meal using an aqueous alkaline bath, forming a liquor. Vegetable protein material is then precipitated from the liquor by acidification. The vegetable protein material is treated with a chelating agent to improve its physical properties. The precipitated vegetable protein material is subjected to an enzymatic hydrolysis treatment resulting in an isolated vegetable protein material having physical properties so improved that the protein mixes with meats to form products with enhanced taste and functional properties.

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The present invention relates primarily to food, and in particular to the preparation of vegetable protein food products. The method of preparing vegetable protein food products of the present invention results in soy protein and similar food protein products which have improved finished meat colour, greatly improved texture, flavour and viscosity when emulsified, and a higher density dry than previously prepared vegetable protein foods. These properties render the protein products more suitable for addition to emulsified meats such as hot dogs, bologna and canned meats than conventional vegetable protein foods.

U.S. Patents No. 3,642,490; 3,694,221 and 2,232,052 disclose products of this general type as well as processes for their preparation.

This invention was conceived and developed largely for soy materials because of the special problems encountered with such soy materials and because of the ready availability of soy products of a good and consistent quality. Therefore, it will be explained largely with respect to soy materials. However it will be apparent that protein meals in general can be used, regardless of their source, and, in particular, vegetable protein meals, such as safflower, cottonseed, sesame, sunflower, peas, oilseed rape and peanut meal, can be used. In its broader aspects the invention includes the use of other vegetable or oilseed materials fish protein materials, and microbial protein products. Accordingly, although the process of the present invention is described, as explained above, primarily with reference to the use of proteins derived from soy, it also applies mutatis mutandis to the use of proteins derived from other materials.

The preparation of vegetable protein products is well known, having been used in a variety of processes. Enzymatic processes for obtaining food meal from vegetable protein materials are also well-known, a typical process being disclosed in U.S. Patent No. 2,232,052. The usual enzymatic process requires a lengthy reaction time, typically several hours at a pH optimum for the enzyme used, and hydrolysis under specific pH and other conditions effective to expose the core proteins of the protein substrate to hydrolysis. The process is conducted as a batch type operation. Within the process for preparing protein foods from, say, soybean meal, various controls are necessary such as a controlled enzymatic reaction to partially hydrolyze, deamidate and modify the protein reactant. The process of this invention may be a continuous type of operation as well as batch. The process results in a product of unexpectedly superior functional properties.

Prior processes for hydrolyzing and/or deamidating, proteins, while producing a commercially useful,product, have not been able to achieve the ultimate in taste and physical properties resulting, on admixture of the product with meats, in a superior meat product. In contrast, the product produced by the process of the present invention can blend smoothly with meat without the need for pre-hydration, and can thereby form excellent tasting products such as hot dogs, bologna and other luncheon meats.

A commercially successful process currently in use for the preparation of isolated vegetable protein materials includes extracting vegetable protein meal, e.g. defatted soybean meal, using an aqueous alkaline bath, to form a liquor. Vegetable protein material is then precipitated from the liquor by acidification. The resulting precipitated vegetable protein material is treated with a polyphosphate to improve its physical properties. Following the polyphosphate treatment the precipitated vegetable protein material is subjected to an enzymatic hydrolysis treatment. In accordance with the present invention, that commercial process has been perfected, resulting in an isolated vegetable protein material having physical properties so improved that the protein can be mixed with meats to form products with enhanced taste and functional properties. The process of the present invention includes the steps of extracting the vegetable protein from the meal with an aqueous alkaline solution but the solution also contains a chelating agent, which can remove by chelation during the protein extraction those elements in the liquor which, through oxidation, are detrimental to colour and odour. Vegetable protein is then precipitated from the solution by acidification. The resulting precipitated vegetable protein is then subjected to an enzymatic hydrolysis under controlled time and concentration conditions for a mild hydrolysis reaction, leading to a change in end groups (reducing the reactivity of the protein material), and to a change in surface characteristics (resulting in a low viscosity when emulsified, and greater density when not emulsified). These two changes yield a vegetable protein of improved taste, odour and colour which in meats form firmer meat products with a more meaty texture.

Thus, the present invention consists in a process for preparing from a protein meal a protein product having physical properties so improved that the protein mixes with meats to form products with enhanced taste and functional properties, which comprises: extracting the protein from the meal with an aqueous alkaline solution, the solution also containing a chelating agent to remove by chelation during the protein extraction elements which through oxidation are detrimental to colour and odour; precipitating protein from the solution by acidification; and subjecting the precipitated protein to an enzymatic hydrolysis under controlled time and concentration conditions for a mild hydrolysis reaction leading to a change in end groups reducing the reactivity of the protein material, and to a change in surface characteristics, resulting in a low viscosity and greater density.

Preferably several extractions, more preferably two extractions, followed by a centrifugation, are effected.

Extensive research has been conducted in an effort to develop useful food products from vegetable

oilseeds. As a result, some of these materials are presently being processed to produce food products commonly called edible vegetable proteins. A widely employed commercially used process for preparing such proteins is given hereafter in Example a.

The chelation step of the present invention, as is shown hereafter in the Examples, results in a great enhancement of physical properties by modifying the protein, particularly colour and taste. Although the use of phosphates, especially polyphosphates, has been described and is preferred, other chelating agents such as ethylenediamine tetraacetic acid, ascorbic acid and citric acid can be used.

Control of the pH during the process may be by use of conventional food grade bases and buffers, such as sodium hydroxide, sodium bicarbonate, ammonium carbonate, sodium tripolyphosphates, hydrochloric acid and other conventional reagents. The optimum pH may differ somewhat for each particular enzyme used, but the process is effective within the pH range of from 5.5, preferably from 6, to 10. More preferably, however, the enzymatic reaction is effected at a pH of from 5.5 to 7.5 and most preferably about 6.5, and it is to a value within this range that the pH of the slurry resulting from the hydrolysis is adjusted. The temperature of the reaction may preferably vary from about 10°C room temperature to about 75°C, and the process is generally effective within this temperature range; although not preferred, temperatures outside this range may also be employed. The optimum temperature conditions may also vary somewhat, within this range, for any particular system.

One of the steps in the formation of the isolated protein herein is the enzymatic hydrolysis step; this is a known step, but it has been found that, under the milder conditions employed in the process of the present invention, the final product is much improved. Such mild conditions include a reduction in the concentration of the enzyme. The enzyme material, rather than being added at a level of from about 0.01 to 5.0% by weight of protein material (dry basis), as is conventional, is preferably used at a level of from 0.01 to 0.15%, and more preferably from 0.01 to 0.035% and most preferably from 0.01 to 0.015%, by weight to achieve mild hydrolysis, depending on the temperature and time conditions employed, and the activity of the enzymes. (Enzyme activity may be defined as the amount of enzyme required to produce a standard amount of tyrosine from casein and maltose from starch under standard conditions.) The time required for the hydrolysis step is preferably from about 5 to 30 minutes, more preferably from about 10 to 15 minutes, depending on enzyme concentration and activity and temperature.

The enzymes which are effective in the process of the present invention are generally those proteolytic enzymes which may be obtained from animal, plant and microbial sources. A variety of enzymes have proven satisfactory. These include papain, trypsin, ficin and a variety of bacterial and fungal proteases. The only limitation on the protease is that it be stable and not be inactivated by the pH used for the process.

The preferred enzyme is a plant protease enzyme, more preferably bromelain.

The reacted slurry is preferably heated to inactivate the enzyme and stop the reaction.

Preferably, following the hydrolysis the protein material is subjected to controlled, rapid, dynamic heating with physical working at an elevated temperature, to produce a 13 to 20 percent solids slurry.

It has also been found that the addition of cysteine increases stability. The use of cysteine herein is an adjunct to the steps of adding polyphosphate to the extraction liquor prior to precipitation, and of mild hydrolysis. Cysteine and other reducing agents can be used to lower and/or reduce oxidation of the extracted curd. In addition the process can include protein isolate with conventional products such as anticaking agents, dispersing agents, antifoaming and antidusting etc. Cysteine can be added in an amount of 0.05 to 0.2 weight per cent based on curd solids to the neutral or the acid curd.

The functional properties, such as taste, texture and handling of commercial materials are not satisfactory for many food uses. The novel protein material produced by the process of the present invention has greatly improved functional properties with better taste, and a particularly good colour for use in meats. The hydrolysis is conducted under mild conditions including a controlled reaction time which is effective in reducing the viscosity and with good texture and flavour.

While the product may be used as a substitute for dairy product derivatives, such as caseinate and milk, it is also much more suitable for mixing with meat than previously prepared isolated proteins. It can be added as a suspension, or the dried powder can be directly added to the meat product. Its excellent smooth texture, mild taste and good colour make this possible. The basic unique product obtained is one of excellent functionality and exceptional handling and mixing properties. The minimization of oxidation, among other changes the polyphosphate and hydrolysis steps bring about, help make this possible.

The invention is further illustrated by the following non-limiting Examples, of which Example a illustrates the prior art.

Example a - Prior Art

(1) Soybeans are ground and the oil is extracted with hexane to yield flakes, which are commonly called

"soybean meal". The flakes are then added to an aqueous bath, and a food grade alkaline reagent, sodium hydroxide, is added until a pH of 10 is reached. The material is extracted for 30 minutes and is then centrifuged. The soy protein material is then precipitated from the liquor by adding hydrochloric acid until the isoelectric point is reached at about a pH of 4.5. The precipitate is then washed with water to make an aqueous slurry of 15% solids by weight.

(2) The pH of the slurry is adjusted to 7.0 by adding sodium hydroxide.

(3) The slurry is then passed through a jet cooker under a pressure of 85 p.s.i.g. simultaneously with steam ejections from the jet cooker under a pressure of 95 p.s.i.g. into a pressure retention chamber at a pressure of 75 p.s.i.g. The steam heats the slurry through the jet cooker to a temperature of 305°F (152°C). After 9 seconds, progressive portions of the heated slurry are suddenly discharged into a receiver at atmospheric pressure or below, causing flash off of vapours laden with undesirable substances. The slurry is cooled by the flash off vaporization. The substance laden vapours are removed from the purified slurry.

(4) The slurry is flash dried in a spray drier to a moisture content of 5% by weight.

In light of this prior art example the process of the invention will now be exemplified in the following non-limiting Examples 1 to 3.

EXAMPLE 1

Ingredient	Amount
soy flakes	100 pounds (45.5 kg)
sodium tripolyphosphate (10% solution)	0.5% based on weight of flakes
sodium hydroxide	as required for pH
Hydrochloric acid	Maintain precipitation pH
Water	1000 pounds (454.5 kg) (First extraction)
	600 pounds (272.7 kg) (Second extraction)
Enzyme (Bromelain)	0.01%

Following the procedure described in Example a, the 100 pounds of soy flakes were added to the aqueous alkaline bath but, in addition, 0.5% of sodium tripolyphosphate was added to the bath prior to addition of the soy flakes. The material was extracted for 30 minutes, centrifuged, and extracted again. The soy protein material was then precipitated as in Example a until the isoelectric point was reached at about a pH of 4.5. The precipitate was washed with water.

Instead of drying the slurry as in Example a, when the slurry temperature reached 125°F (52°C) (the pH of the material being 6.5), the aqueous solution of Bromelain enzyme was added at the low level of 0.01% by weight of protein (dry basis). The enzyme treated slurry was allowed to react for fifteen minutes as the viscosity of the slurry began to drop.

After fifteen minutes, the reaction of the enzymes was stopped by raising the temperature. The slurry was then flash dried in a spray drier to a moisture content of about 5 weight percent as in Example a.

Treating the protein liquor during the extraction steps, that is before precipitation, brings about a release and removal of undesirable compounds to improve the physical properties such as colour, odour and stability. The mild enzymatic hydrolysis modifies the isolated protein product by changing end groups and surface characteristics to bring about a superior product with better texture and a more meaty flavour.

Properties of an isolated soy protein prepared by the process of Example 1 are set forth in Table 1.

Table 1

Isolated Soy Protein Evaluation

Total Solids (%)(water slurry)	9.0
pH	6.3
Protein (%AI)	86.6
Protein (%DB)	90.5
Moisture (%)	4.3
Ash (%).7	5.1
Sulphite (ppm)	2
Iron (ppm)	114
Trypsin Inhib. (TIU/mg).4	7.8
End-groups (mNH2/g sam)	4.4
Density (lb/ft3)	15.4
Gel Rate (g, 10min)	67

Meat System French Frankfurter

Fracture Force (g)	6453
Hardness (g)	7151
Stability (g/100g)	5.2
Yield (%)	89.4
pH5	6.2
Hunter L	52
a	8.8
b	8.3

In order to compare the product prepared according to Example 1 with the commercial product prepared by Example a the following test data using a conventional meat system is given in Table 2. The meat system included a beef frankfurter formula containing 37% high quality meat, 13% organ or mechanically deboned meat, and 5.7% assorted flours and starches. The isolated soy protein is present at a level of 2.41%.

Table 2

	Example a	Example 1
Fracture Force (g)	2773	3784
Hardness (g)	5307	5469
Emulsion Stability gm/100	0.99	0.35
Yield %	92.1	92.0
pH	6.05	5.93
L	44.8	45.3
a	12.7	12.5
b	28.3	8.1

Sensory Texture

Commercial Test

pH	5.75	5.72
L	46.3	44.7
a	13.1	13.5
b	9.7	9.5
F x (fracture force, g.)	2268	2482
s.d.	221	330
H x (hardness, g.)	4717	5145
s.d.	385	769

The data in Table 2 show that, by using the polyphosphate in the extraction step, a superior product can be made. The isolated soy protein of the invention is more firm and is much more stable than that of Example a. Stability, for instance, is 0.35 compared to 0.99. In addition the product of the present invention has better colour for emulsified meats, and a lower soy flavour. It also has a more meaty flavour and superior texture.

EXAMPLE 3

Following the procedure of Example 1, additional proteins were prepared using hexametaphosphate in the extraction water instead of sodium tripolyphosphate. There was no significant difference in properties and the finished meat had the same improved colour and taste.

Having been given the controlling features of the invention modifications will occur to those in this well known, but still empirical art. Thus sulphites, defoaming agents and the like can be included in the formulations. And it has already been indicated that pH values and temperatures can be varied. Such ramifications are deemed to be within the scope of the invention. It is intended that the invention not be restricted to the embo-

diments given herein for purposes of illustration, but is to be limited only by the claims appended hereto and their equivalents.

Claims

1. A process for preparing from a protein meal a protein product having physical properties so improved that the protein mixes with meats to form products with enhanced taste and functional properties, which comprises: extracting the protein from the meal with an aqueous alkaline solution, the solution also containing a chelating agent to remove by chelation during the protein extraction elements which through oxidation are detrimental to colour and odour; precipitating protein from the solution by acidification; and subjecting the precipitated protein to an enzymatic hydrolysis under controlled time and concentration conditions for a mild hydrolysis reaction leading to a change in end groups reducing the reactivity of the protein material, and to a change in surface characteristics, resulting in a low viscosity and greater density.

2. A process according to Claim 1, in which the protein meal is a vegetable protein meal.

3. A process according to Claim 2, in which the protein meal is a vegetable protein meal derived from an oilseed.

4. A process according to Claim 3, in which the vegetable protein meal is safflower meal.

5. A process according to Claim 3, in which the vegetable protein meal is soybean meal.

6. A process according to any one of the preceding Claims, in which several extractions followed by a centrifugation are effected.

7. A process according to Claim 6, in which two extractions followed by a centrifugation are effected.

8. A process according to Claim 1, in which following the hydrolysis the protein material is subjected to controlled, rapid, dynamic heating with physical working at an elevated temperature, to produce a 13 to 20 percent solids slurry.

9. A process according to any one of the preceding Claims, in which the pH of the slurry resulting from the hydrolysis is adjusted to between about 5.5 and 7.5.

10. A process according to Claim 9, in which the pH of the slurry resulting from the hydrolysis is adjusted to about 6.5.

11. A process according to any one of the preceding Claims, in which the enzyme is an animal, plant or microbial enzyme.

12. A process according to any one of the preceding Claims, in which the enzymatic hydrolysis treatment is effected with a plant protease enzyme.

13. A process according to Claim 12, in which the plant protease enzyme is bromelain.

14. A process according to any one of the preceding Claims, in which L-cysteine is added to the precipitated soybean material being hydrolyzed to increase product stability.

15. A process according to any one of the preceding Claims, in which the enzyme is added at a level of from 0.01 to 0.15 percent by weight based on the dry weight of the protein in the slurry.

16. A process according to any one of the preceding Claims, in which the reacted slurry is heated to inactivate the enzyme and stop the reaction.

17. A process according to Claim 16, in which the slurry is flash cooled, producing a dry product weighting 16 to 20 pounds per cubic foot.

18. A process according to any one of the preceding Claims, in which the chelating agent is a phosphate, preferably a polyphosphate.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 30 6581

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	EP-A-0 141 615 (RALSTON PURINA CO.) * Claims 1-21; page 5, lines 20-25; pages 6-13 * ---	1-17	A 23 J 3/34 A 23 J 1/14
Y	WPIL/DERWENT, accession no. 77-13723Y [08], Derwent Publications Ltd, London, GB; & JP-A-52 003 844 (MITSUBISHI GAS CHEM. IND.) * Abstract * ---	1-17	
A	EP-A-0 148 600 (RALSTON PURINA CO.) * Claims 1-19; page 5, lines 15-25; pages 6-15 * ---	1-17	
A	WPIL/DERWENT, accession no. 77-04733Y [03], Derwent Publications Ltd, London, GB; & JP-A-51 139 650 (MITSUBISHI CHEM. IND. K.K.) * Abstract * -----	18	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 23 J

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-10-1991	SANTOS Y DIAZ A.I.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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